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Parasitism of *Armillaria mellea* in Relation to Conifers

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By W. R. DAY, M.A.
(Imperial Forestry Institute, Oxford)

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THE PARASITISM OF *ARMILLARIA MELLEA* IN RELATION TO CONIFERS.

By W. R. Day, M.A. (*Imperial Forestry Institute, Oxford*).
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Armillaria mellea, the honey fungus, is too well known to need any introduction. In conifer plantations in this country it almost invariably causes some loss, and under some circumstances the loss may be very great. As to what these circumstances really are is, perhaps, still a matter for investigation. In this paper I propose first of all to attempt to show, from observations made on attacked trees, the manner in which the fungus penetrates the host; then I wish to discuss shortly the susceptibility of different species of conifers of importance to forestry in this country, and in connection with this, some of the possible reasons for such susceptibility.

So far as I know, no really satisfactory work has yet been done to show the manner in which *A. mellea* enters its host. Brefeld made inoculation experiments on pieces of living pine root and found that the fungus easily penetrated the cork layer. The condition of such a piece of root is so different from that of a root still attached to the tree that very little of practical importance can be learned from the experiment. Neger described rhizomorphs piercing the bark of silver firs, the tops of which were dying back, but he considered that in this case the fungus was only entering a host already dying and therefore in a very weakened condition. Now, observations show that honey fungus is no respecter of persons, and that the less thrifty trees are not the only ones attacked. A statistical study might show that more trees of this type are

attacked than those that are thrifty and well grown; of that I can say nothing, but it is definitely true that often among the trees to die of honey fungus or to be attacked by it are some of the best and strongest growing. Upon uprooting, such trees are found to have a well-developed root system which apparently is in every way healthy, but for the attack. The question arises, how are such trees entered by the fungus? Does it enter through wounds, or dead roots only, or can it pierce the cork layer of an uninjured root? If it is able to do this it would appear to have some claim to be called a virulent parasite of primary importance. As to how far such a claim is justified is discussed later.

The evidence that I have to put forward shows the manner in which honey fungus attacks healthy, vigorous trees. It would perhaps have been better had it been collected from such trees specially inoculated with, or exposed to, attack by a pure culture of the fungus. Such experiments are difficult to carry out satisfactorily under natural conditions, and so far there has been no opportunity of attempting them. The trees from which the evidence has been taken belonged to several different species and genera, and the occurrence of the same type of phenomenon in each case shows that the method of attack does not vary from host to host.

The first stage of attack consists in the attachment of the mycelium of the fungus to the bark of the host. In all cases the fungus appears to attack the host solely by means of its rhizomorphs. There is no need to describe these: they are sufficiently well-known already. They are to be found, as a rule, within the top six inches of the soil; very occasionally they run deeper, or come up from some root that is more deeply buried.

The rhizomorphs become definitely attached to the host, quite apart from penetration. If they reach a part of the host where there is a thick layer of dead bark, as at the root collar of a pine, they enter the dead tissues and form a mycelium therein. This may occur without any penetration of the tree taking place; but should such happen it is done by one of the rhizomorphs that develop as a

Fig. 1

Rhizomorph attached to the outer cork layer. The dotted line indicates the remains of decayed cork tissue.

Fig. 2

A = Rhizomorph. B = Old cork layer. C = Resin. D = New cork layer with starch filled cells behind it.

Fig. 3

A = Old cork layer. B = New cork layer with starch filled cells behind it. C = Rhizomorph.

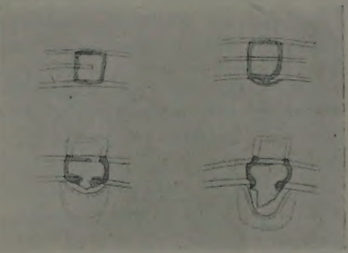

Fig 4

Sections through Rhizomorph at and near point of entry of a Jap. larch root. For explanation see text.

part of this mycelium. If the host is smooth barked, as, for example, are young larch, Thuja or Douglas fir, or the roots of any tree, the rhizomorph becomes attached to the outside of the host, and this attachment takes place by one side of the rhizomorph definitely growing into and forming in the dead outer cork cells (Fig. I.), and also probably by the root-hair-like hyphæ that surround the rhizomorph tip penetrating these cells without the rhizomorph growing into them. The attachment is so firm that it often takes much force to rip a rhizomorph away from the part it is attached to. A favourite place of attachment is the root-collar. Small trees are quite frequently encircled with rhizomorphs at this point, and when so encircled may be gripped so tightly that the bark is furrowed, exactly as if a piece of string or wire had been tied round so as to allow no space for further growth.

A secondary means of attachment, as also of penetration, is by rhizomorphs, which branch off from the main one at the point of attachment. This frequently occurs; for example, where a rhizomorph tip directly meets the side of a host, a whorl of such branches may develop and all become attached to the host.

The mere attachment of a rhizomorph to a possible host does not, of necessity, involve infection. Whether or not infection follows depends on conditions largely unknown.

As a precedent to penetration, the fungus appears to exert a toxic influence on the tissues of the host. This can only be detected in the very early stages of attack, but sometimes it is then fairly obvious. Fig. 2 illustrates this. The fungus is here represented as growing in the bark at the root collar. Rhizomorphs press up against the most recently formed cork layer and are in process of penetrating it. On the far side of the cork layer the cortical cells have become empty of contents, are dead or dying, and are being cut off by a new cork layer, behind which starch is massed in great quantities. Fig. 3, made from another part of the same tree, shows a similar state of affairs, but here the section does not go through the point of the rhizomorph. These drawings were made from sections cut from a *Pinus rigida*. Nowhere had a rhizomorph penetrated

through the last formed cork layer and the only sign of attack was a slight resin flow at the root collar. The drawings show, therefore, a very early stage of attack; they clearly demonstrate the toxic effect of the fungus upon the tissues of the host, in advance of its mycelium, and suggest that this influence is exerted even through a cork layer, but this will probably be considered to need confirmation.

A very similar condition is seen in an early stage, just after penetration has happened. The diagrams shown in Fig. 4 represent sections cut more or less in series through, and to one side of, the point of penetration. Several old cork layers appear embedded in the rhizomorph; the last one to be penetrated shows no particular sign of mechanical rupture but appears as though dissolved away. The point of the rhizomorph extends but a little way into the tissue; the cells immediately below have brown walls which appear to be thickened or swollen and also to be slightly crushed. Below them is a small but distinct zone of cells from which most of the contents are withdrawn, while below this zone appears the normal cortical cell tissue. No hyphæ were discovered beyond the point of the rhizomorph, so that here again the toxic influence of the fungus in advance of the mycelium distinctly appears.

Evidence as to the condition of the host at the time of penetration is found in the arrangement of cork layers around the point of entry of the rhizomorph. If the host is living and possessed of any considerable vitality it almost always forms one or more secondary cork layers in order to prevent, if possible, the entry of the parasite. Sometimes, however, no such cork layers can be seen, and then one has the case of simple and direct penetration without any other let or hindrance than that provided by the original outer cork layer. Fig. 5 illustrates this. Here the rhizomorph has grown in the outer dead cells of the cork layer and has broken through and pushed back the others. It is not possible to tell from such a case whether or not the stem was living or dead at the point of entry when the rhizomorph pushed through. The section shown was cut from the stem of a young and living Douglas fir;

neither here nor elsewhere was there any sign of mechanical injury, nor is there any particular reason to suspect it. Many such cases are not simple in reality; what has often happened is that the outer cortical tissue and cork layers have become decayed and fallen off, and probably this is what has happened here.

In the more complex type of penetration, secondary cork layers are always apparent. One distinct feature in many cases is the formation of deep invaginations, an example of which is shown in Fig. 6. This is from a Corsican pine, a dominated tree but one that was still growing well. The invagination through which the rhizomorph has penetrated is lined with a series of cork layers; these were broken through eventually and a last attempt to keep out the fungus was made in the formation of a further layer deeper in the cortex. This layer was distinguished from the others by the unthickened cell walls, which indicates its more recent formation. The cork layers lining the invagination were really formed within the cortex, but the outer tissues have fallen away in this section. This invagination was only one of a number formed at this place by the rhizomorph and its branches, and only through this one and one other was the fungus successful in penetrating.

A much earlier state in the formation of an invagination was found in a Norway spruce, in this case a dominant tree. The invagination was of the type shown in Fig. 6, but the cork layers were still surrounded by living cortex and the fungus had not yet reached the cambium. Penetration in this case occurred at the root collar; a typical fructification grew from one of the rhizomorphs, which appear to have bored straight into the uninjured bark of the tree.

Another example of invaginations is shown in Fig. 7, taken from a young Japanese larch. Here two are shown. The fungus has penetrated through the shallower one, and a branch rhizomorph went down the other. A fragment of cork layer is shown in the middle of the rhizomorph. Here again the cork layer seen is not the normal outer one, but was formed within the cortex to keep out the fungus.

Fig. 5

Cork layer of Douglas Fir broken through by *Armillaria mellea* rhizomorph (shown shaded).

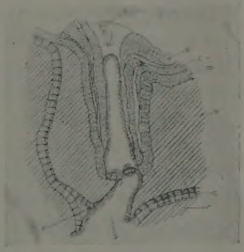

Fig. 6

A = Resin with cork cells embedded in it. B, C, D and E = Cork layers. F = Cortex and G = Rhizomorph. At H there is resin accumulated where E is broken. The cambium is below the section.

Fig. 7

Jap. larch root shewing point of entry of rhizomorph, invaginations, resin cavities near to point of entry.

Fig 8

Jap. larch root shewing point of entry of rhizomorph with cork layers embedded in it.

Such invaginations are not found necessarily. Fig. 8 shows a case where penetration has occurred without one being formed.

Even when there is no sign of secondary cork layers there is often evidence of abnormal growth (Fig. 9). In this section of a Japanese larch root the cortex is seen to be greatly enlarged on one side. This eccentric growth occurred only where the rhizomorph had penetrated: elsewhere the root was circular. Now the growth must have occurred before penetration, for by then the fungus must have killed the tissues in advance of its mycelium, and the enlargement of the cortex would have been impossible. The enlargement is not due to the growth of the fungus within the cortex, for in passing through the remainder of the root no distortion from the normal circular shape occurred; also the cork layer shows no sign of distortion. This seems to indicate quite definitely that the root was alive at the time of attack, and, since there is no evidence to the contrary that at least the cork layer shown was uninjured.

The conclusion to be drawn from the evidence put forward is, I think, that *A. mellea* is able to penetrate an uninjured and apparently healthy host. Accordingly it might appear that the fungus is a virulent parasite and a primary cause of disease. This is by no means certain, however, if for one reason only, and that, because it is possible sometimes to find trees that are attacked by the fungus and yet do not die, or that, belonging to a species reckoned to be susceptible, are in close proximity to the parasite and yet show no signs of successful attack. This leads inevitably to the general question of the susceptibility of species.

It has always been obvious that some species or genera are generally more susceptible to attack than are others. The actual order in which species are classified as susceptible probably varies according as the experience of foresters differs. Susceptibility itself may be of more than one type; some species are susceptible to attack and yet do not die, whereas others appear almost invariably to die after attack. The order of susceptibility of the more important conifers in Bagley Wood is somewhat as follows:—

(a) *Susceptibility to attack, without considering liability to death afterwards* :—

1. *Pinus spp.*
2. S. Spruce.
3. *Thuja plicata.*
4. *Tsuga heterophylla.*
5. N. Spruce.
6. E. and J. Larch.
7. Douglas Fir.

(b) *Susceptibility to death after attack* :—

1. *Pinus spp.*
2. S. Spruce.
3. N. Spruce.
4. E. and J. Larch.
5. *Tsuga heterérophylla.*
6. *Thuja Plicata.*
7. Douglas Fir.

If a wider area were considered the orders here shown would probably have to be altered somewhat.

Now several things have led me to believe that the susceptibility of species may be determined by external factors at least as much as by any inherent qualities. Two or three examples may be taken from the genus *Pinus*, a genus that is generally acknowledged to be among the most susceptible. In Bagley Wood I found a Corsican pine, a dominant tree about 22 years old, into the thick bark of which, about the root collar, *A. mellea* had entered and formed mycelium; this mycelium had even begun to send out new rhizomorphs into the soil. The Corsican pine is one of the most susceptible species in Bagley, yet there was no sign that this tree was being attacked.

Then again, I examined a plantation in which Scots pine and Japanese larch grew side by side, under apparently similar conditions. The trees had been planted for three years, and were making good growth. Very few indeed of the pines had died through attack by *A. mellea*, yet between 5 and 10 per cent. of the larch were dead or dying, and these included some of the best grown and developed trees. The

examination of a large number of larch trees attacked but still living failed in the great majority of cases to show any sign of a wound at the point of entry of the fungus; indeed, everything indicated that the roots were sound at this point when attacked. Now this larch is usually reckoned to be a much less susceptible species than is Scots pine; further, while it may have existed, no other cause could be found as the primary reason for the dying back.

A third example is from N. Devon, where I saw Scots pine on a site in which *A. mellea* rhizomorphs were very abundant. The trees were 16 years old, and but very few indeed had been lost due to attack by honey fungus.

These examples concern instances where a susceptible species is for some reason resistant, although it is known that a virulent strain of the fungus is present.

Further evidence as regards susceptibility is to be found when resistant species are attacked, but do not die back.

In the plantation referred to above, a few Japanese larch trees were found which had been attacked, but showed every sign of being on the way to make a good recovery.

Then, on the more resistant species cankers form at soil level due to attack by the fungus (Figs. 10 and 11). So far none has been found on susceptible species, such as the pines. The species on which they have been discovered are :—*Thuja plicata, Tsuga heterophylla,* European and Japanese larch, Lawson's cypress and Douglas fir. They almost certainly occur on Norway spruce and probably on other species too. Of the cankers examined it may be said : (a) they always occur at the base of the stem, and (b) are connected with one or more dead roots (this not necessary); (c) the tap root has always been dead and infected, (d) as in some cases were also the other deep-going roots; (e) the surface humus feeding roots were always the last to remain alive; (f) some, but not all of the cankers started or enlarged after 1921; (g) many cankered trees show no signs of dying, (h) but if at all suppressed they often die probably much sooner than would otherwise

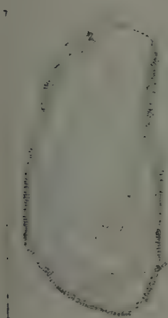

Fig. 9

A. mellea in Jap. larch root shewing enlargement of cortex at point of entry.

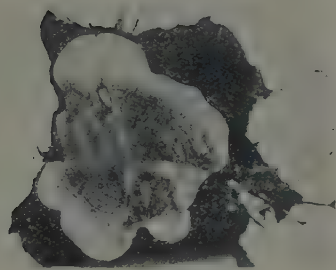

Fig. 10

Canker on *Thuja plicata* due to *A. mellea.*

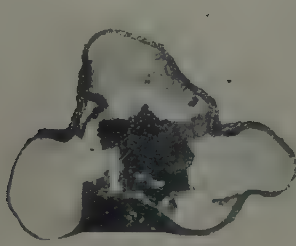

Fig. 11

Canker on *Tsuga heterophylla* due to *A. mellea.*

have been the case; (i) the suppressed and dominated trees are not especially cankered; the dominant ones are frequently attacked; (j) the decay of wood is limited, being restricted to the bottom two to three feet of the stem; (k) this indicates that *A. mellea* is not one of the true wood-rotting fungi, such as *Fomes annosus*.

The formation of cankers in this manner cannot be said to be a sign of a very virulent parasite. Rather, it indicates one that takes advantage of conditions of environment which affect the host unfavourably. The formation of canker after drought is an example of this.

What has been said on susceptibility may be summarised as follows:—

(a) Certain species and genera of conifers appear fairly consistently to be more susceptible to attack by *A. mellea* than are others.

(b) Instances can be found, however, in which one of the more susceptible species is less attacked than one usually resistant.

(c) Moreover, susceptible species growing in close proximity to an abundant growth of the fungus sometimes are not attacked.

(d) Also, those species generally reckoned among the more resistant are sometimes attacked but not killed, as is shown by the formation of cankers at the base of the stem.

The general conclusion that is to be drawn from what has been brought forward is, I think, that while some species or genera may be inherently more susceptible to attack than others, and perhaps it may be proved that even within a single species one race is more particularly susceptible than other races, other and external factors often, and perhaps always, have a much greater influence in determining the intensity of infection and general susceptibility of species. Some of these factors may lie in the fungus; under some conditions it may be more virulent or different races of the parasite with varying virulence may exist; that has yet to be proved. On the other hand the remaining factors of environment, such as the general

climate, the condition of the soil, the silvicultural conditions in general, may be more responsible for the variations in susceptibility to be observed. Sometimes such factors are obvious; the abundant development of rhizomorphs that occurs on felling a broad-leaved wood plainly makes the infection of the next crop very probable; and the death of trees, due to attack by fungus, after a period of drought needs no explanation. Such obvious things as these do not really go to the root of the matter. What has to be asked is—what are the real climatic and edaphic conditions necessary to each species; in what way do we at present observe these conditions, and to what extent does our present type of silvicultural management succeed or fail to make such conditions possible? Eventually the problems of root fungi such as *A. mellea* must be attacked along these lines. That is to say, what is wanted is the development, for the conditions existing in this country, of a silvicultural practice based upon a real knowledge of the requirements of the crop on the one hand and of its possible parasites on the other, and it is with regard to one of the latter that I have attempted to make some small contribution.